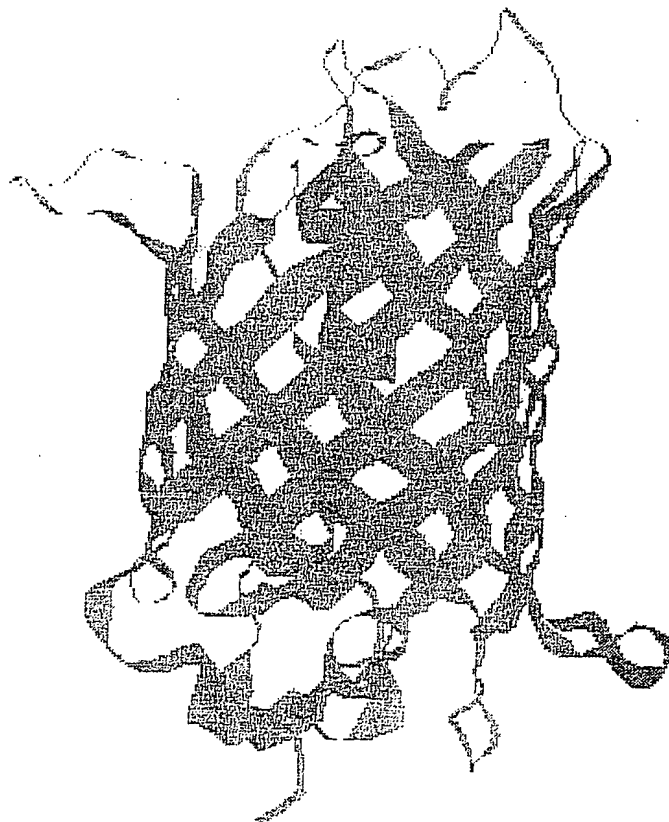


008220" E8E/2960



concept - binding to fluorescent protein
changes fluorescence spectrum

goal - convert the Green Fluorescent Protein
into a universal biosensor, afFP

Figure 1A

000220" 08E22060

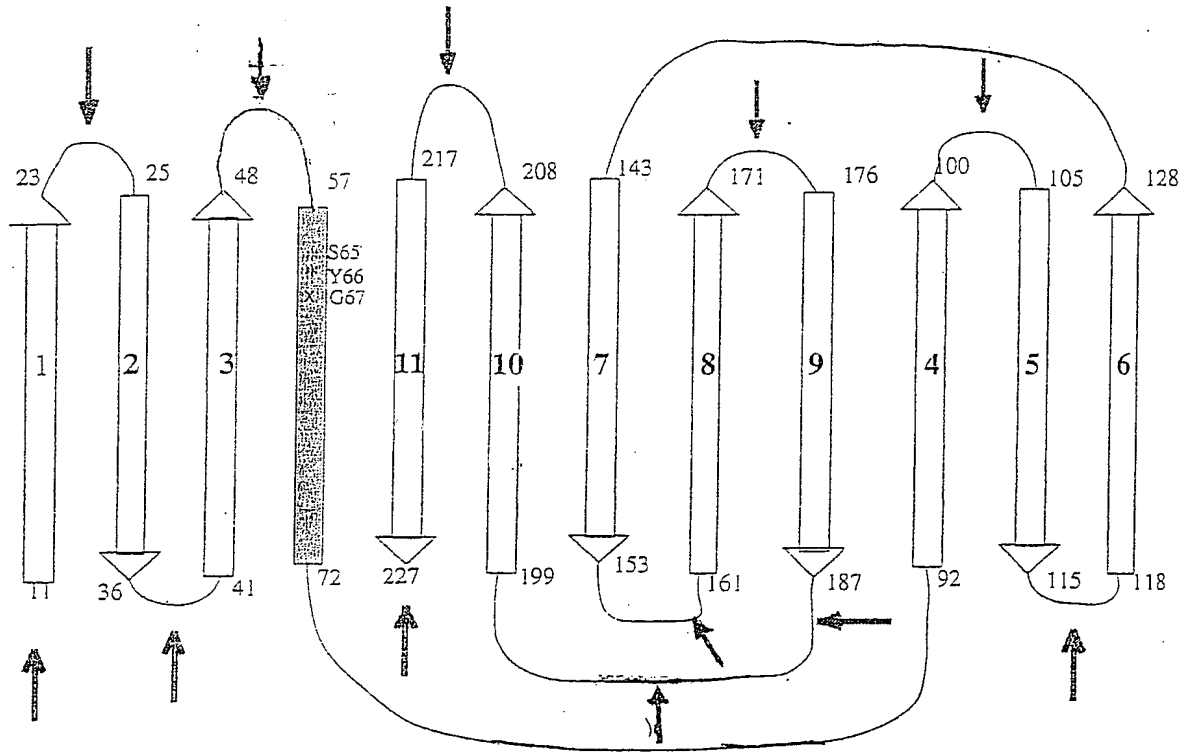


Figure 1B

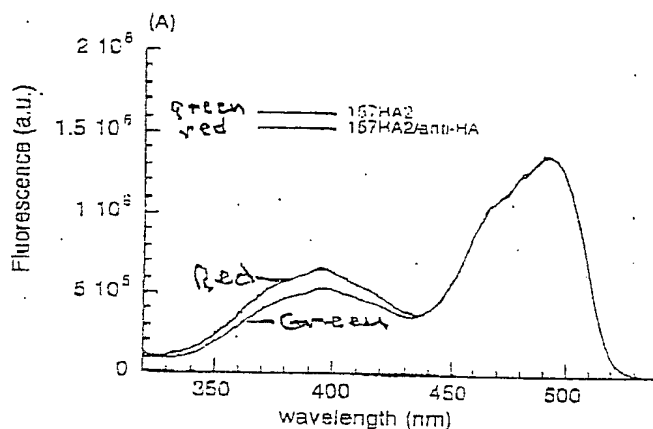


Figure 2A

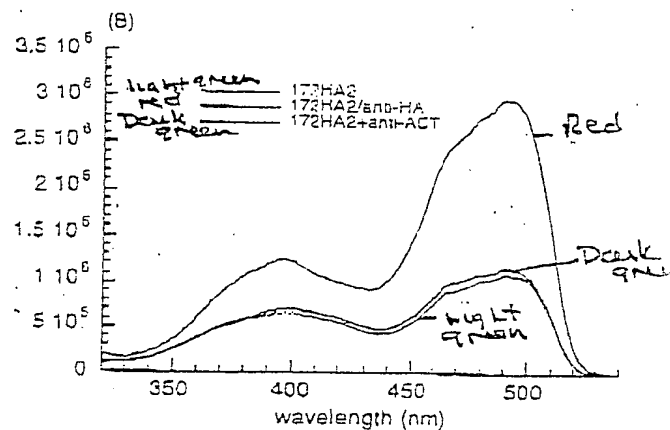


Figure 2B

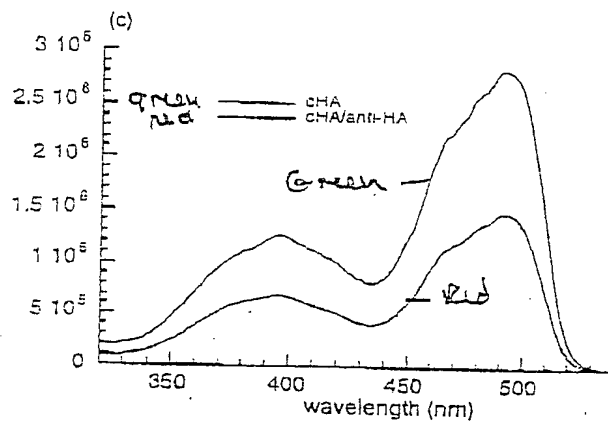


Figure 2C

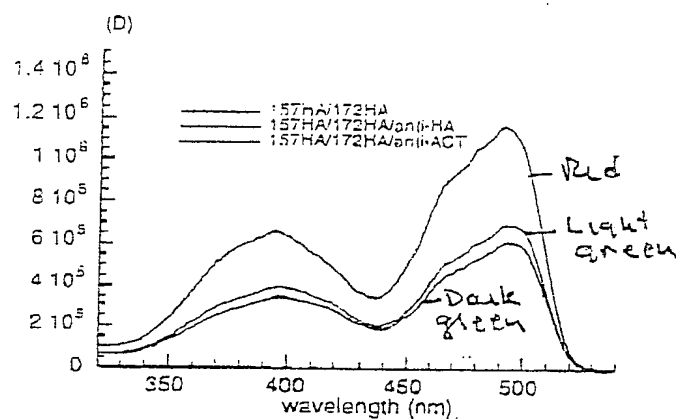


Figure 2D

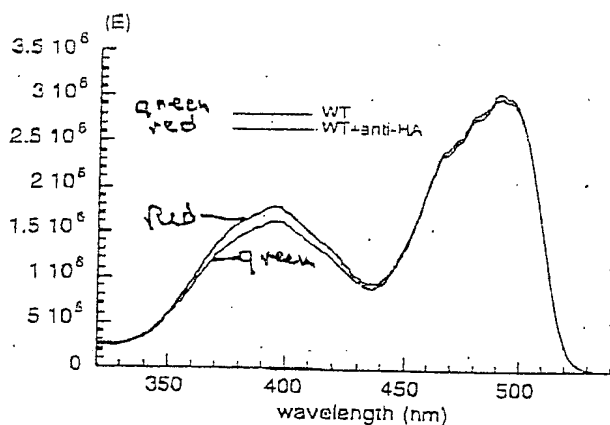


Figure 2E

Excitation spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, and anti-ACT (monoclonal antibody against antichymotrypsin) were 0.3 mg/ml, 3.4 mg/ml, 3.4 mg/ml respectively. The spectra were collected at fixed emission wavelength of 550 nm. The red lines represented complexes. The green lines represented mutants alone. The blue lines represented mutants plus an nonspecific antibody as negative control.

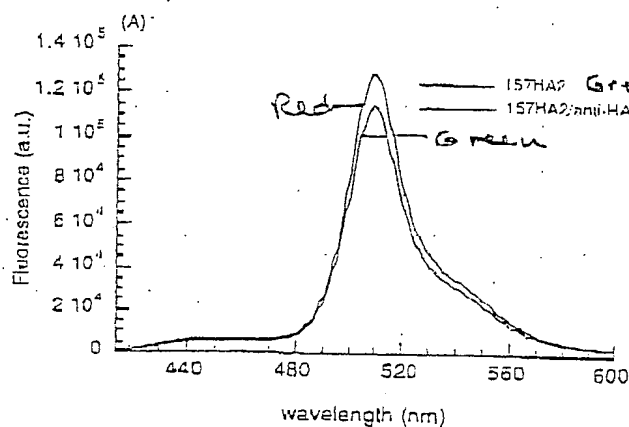


Figure 3A

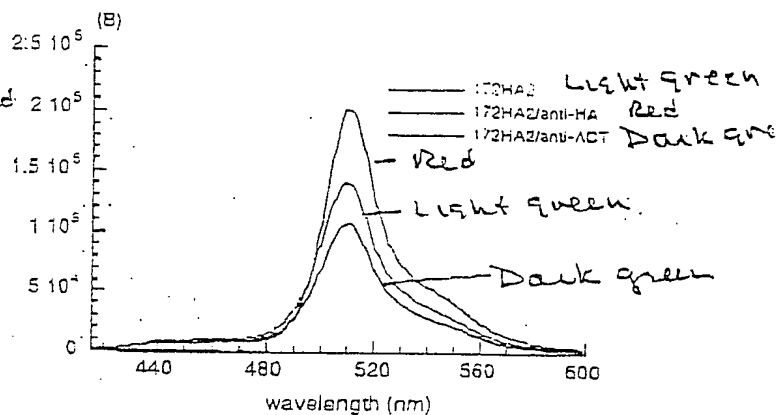


Figure 3B

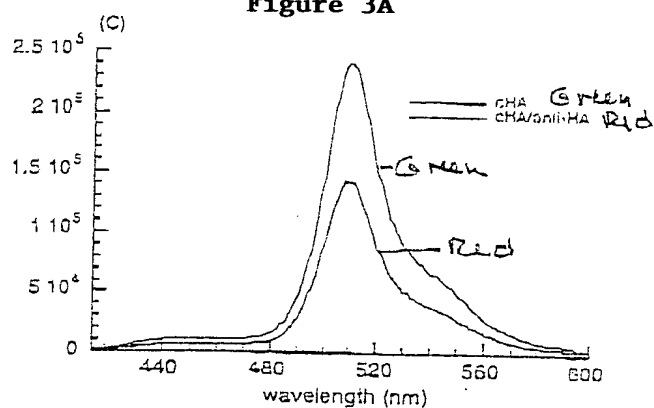


Figure 3C

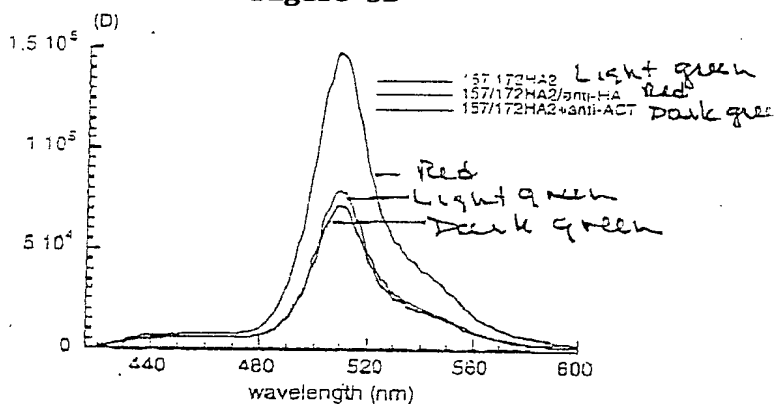


Figure 3D

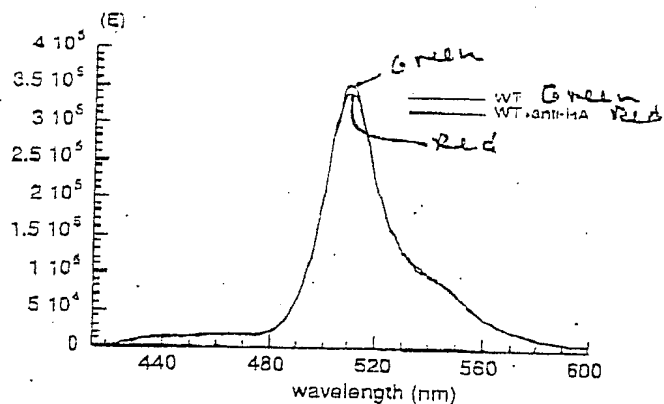


Figure 3E

Emission spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, and anti-ACT (monoclonal antibody against antichymotrypsin) were 0.3 mg/ml, 3.4 mg/ml, 3.4 mg/ml respectively. The spectra were collected at fixed excitation wavelength of 395 nm. The red lines represented complexes. The green lines represented mutants alone. The blue lines represented mutants plus an nonspecific antibody as negative control.

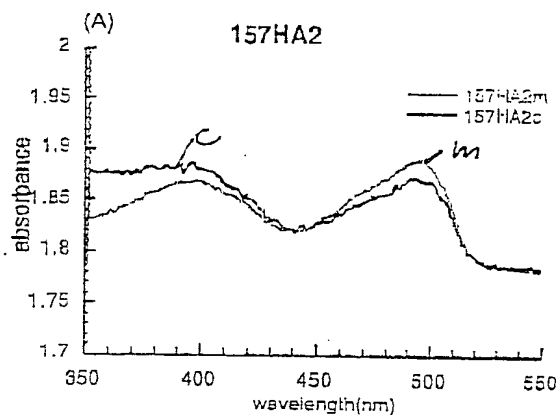


Figure 4A

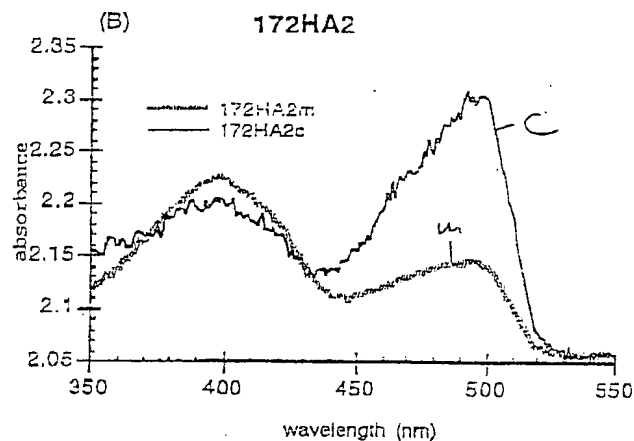


Figure 4B

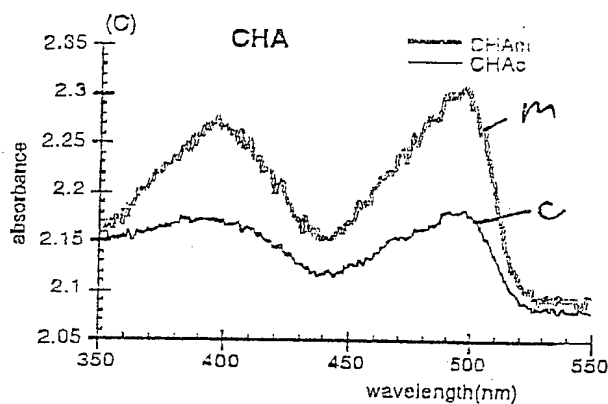


Figure 4C

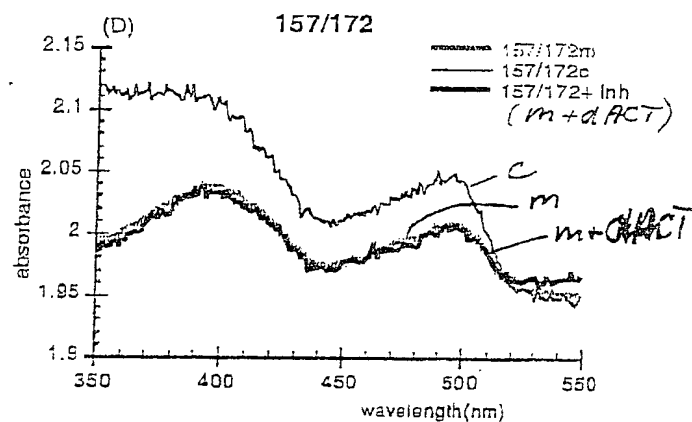


Figure 4D

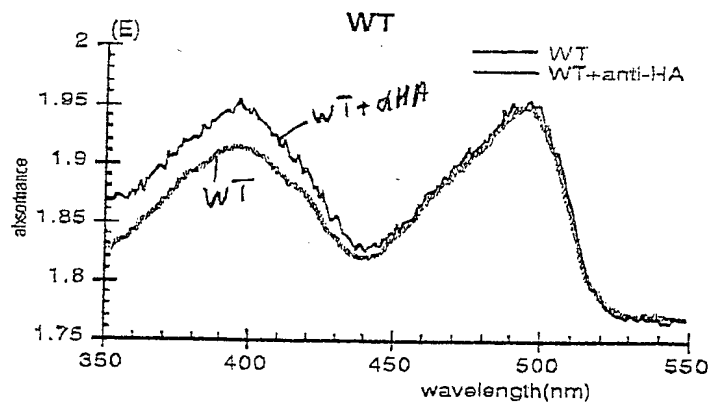
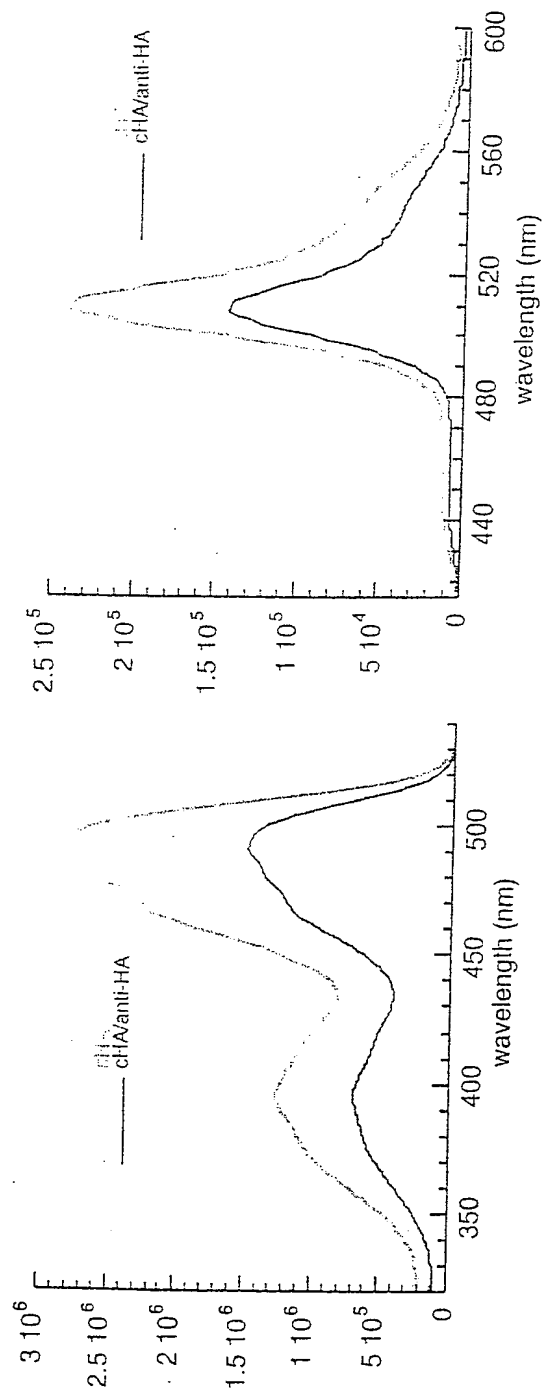


Figure 4E

Absorption spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, and anti-ACT (monoclonal antibody against antichymotrypsin) were 0.3 mg/ml, 3.4 mg/ml, 3.4 mg/ml respectively. The red lines represented complexes. The green lines represented mutants alone. The blue lines represented mutants plus an nonspecific antibody as negative control

Enhanced fluorescence upon binding



excitation

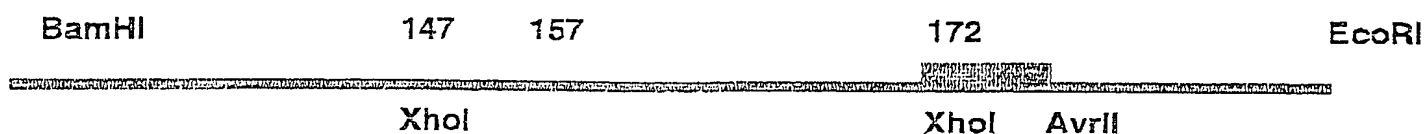
emission

Figure 5

157HA



172HA



157HA2



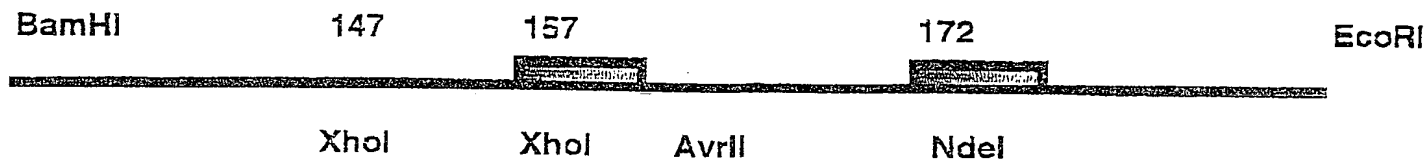
157cyHA2



Figure 6A

008270"EBE/2960

157HA/172HA



172HA2



157HA/CHA

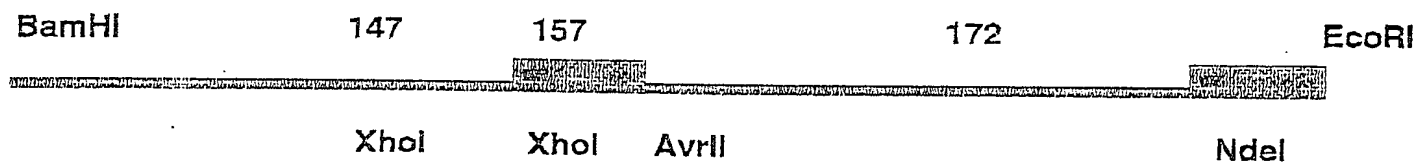


Figure 6B

008240" E8E72960

The diagram illustrates the binding of a ligand to a protein (aFP) to form a complex with different fluorescence. On the left, the protein (aFP) is shown as a cylinder with a white rectangular patch on its side. A black dot on the protein is labeled "Molecular recognition site" with an arrow. Below the protein is the label "aFP". In the center, a plus sign (+) is followed by a circle representing the "Ligand". An arrow points from the ligand to the right, where the resulting "aFP-ligand complex" is shown. In this complex, the protein's rectangular patch is black, and the ligand (circle) is bound to the molecular recognition site. Below this complex is the label "aFP-ligand complex with different fluorescence".

Figure 7

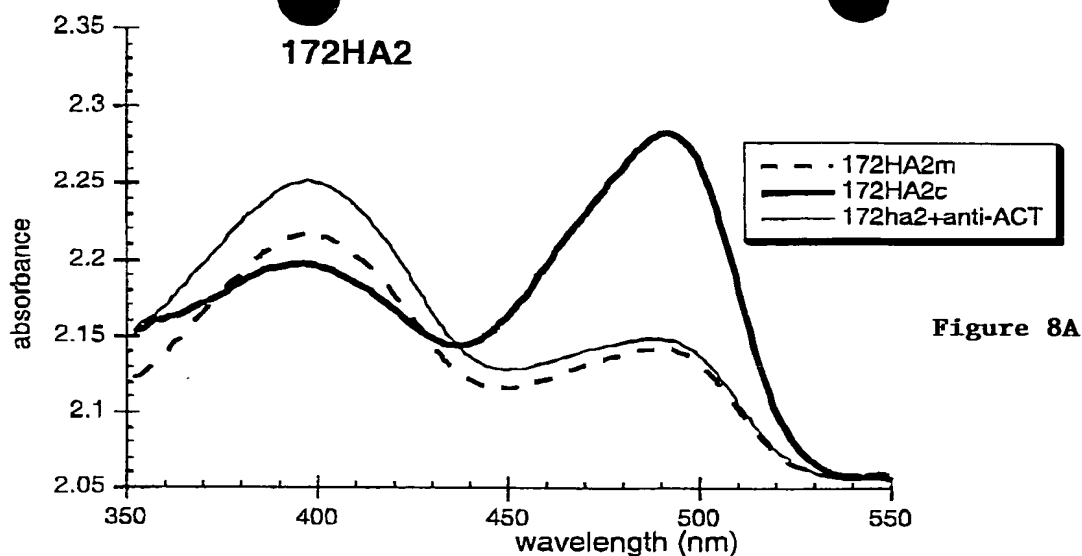


Figure 8A

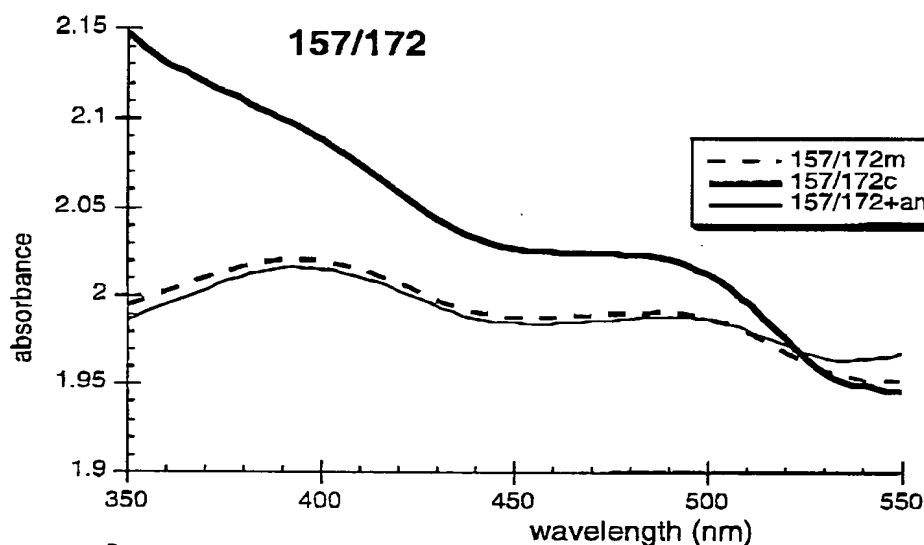


Figure 8B

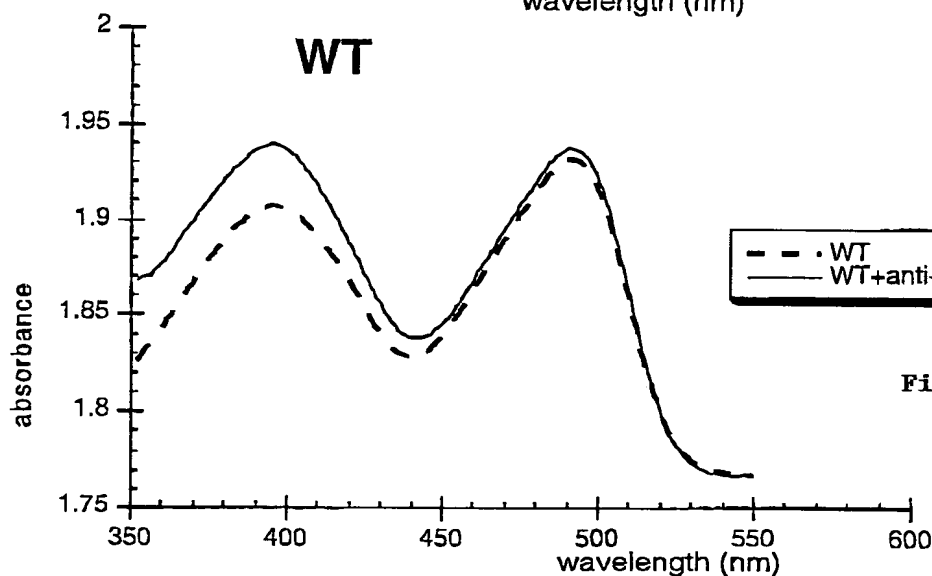


Figure 8C

Absorption spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, and anti-ACT (monoclonal antibody against antichymotrypsin) were 0.3 mg/ml, 3.4 mg/ml, 3.4 mg/ml respectively. The thick lines represented complexes. The dashed lines represented mutants alone. The thin lines represented mutants plus a nonspecific antibody as negative control

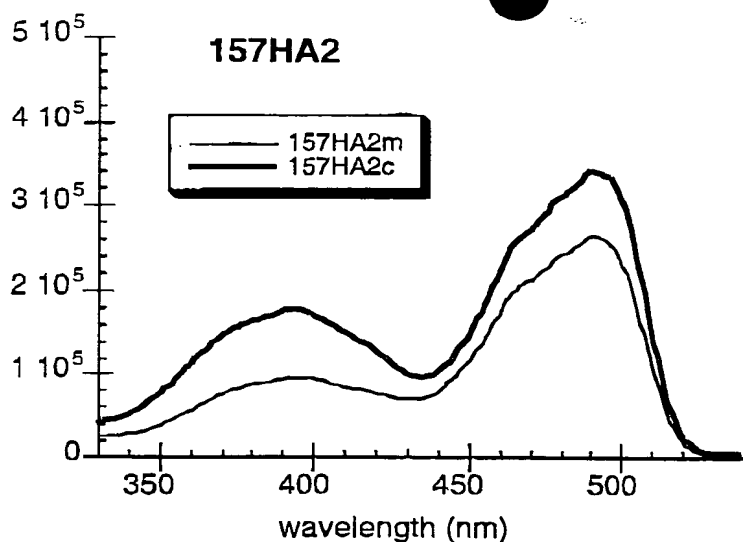


Figure 9A

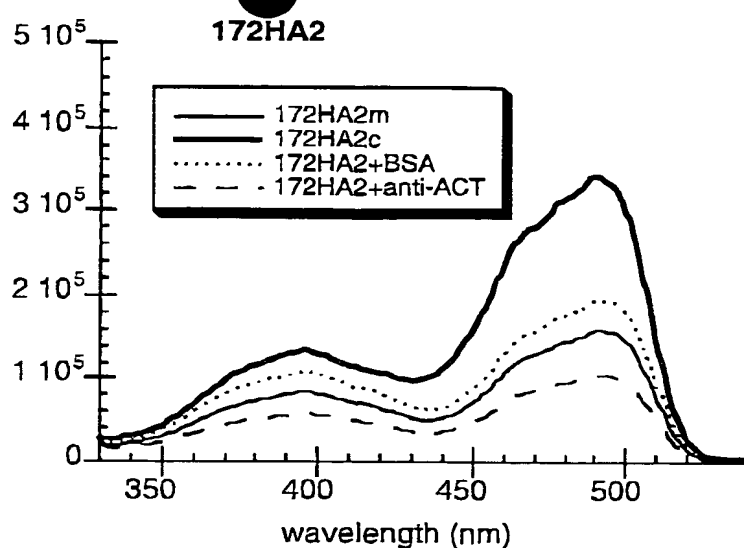


Figure 9B

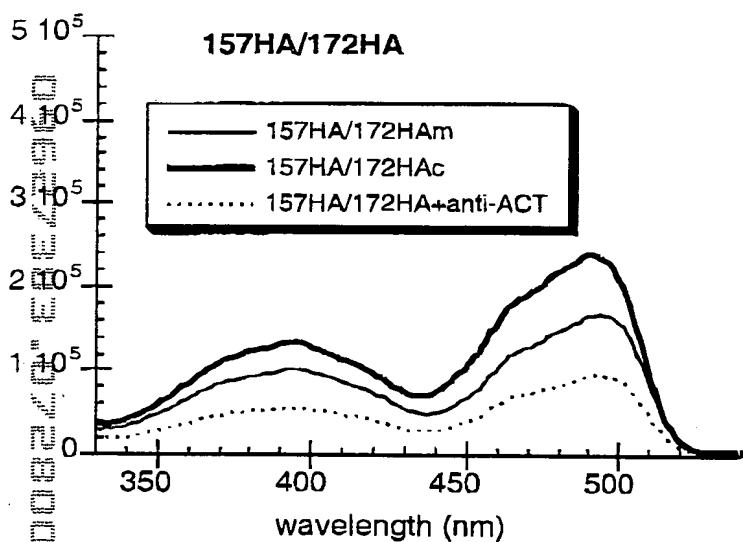


Figure 9C

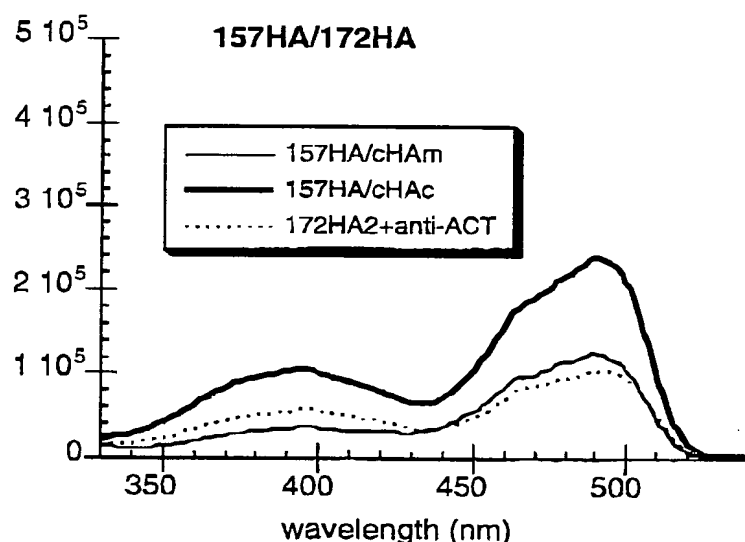


Figure 9D

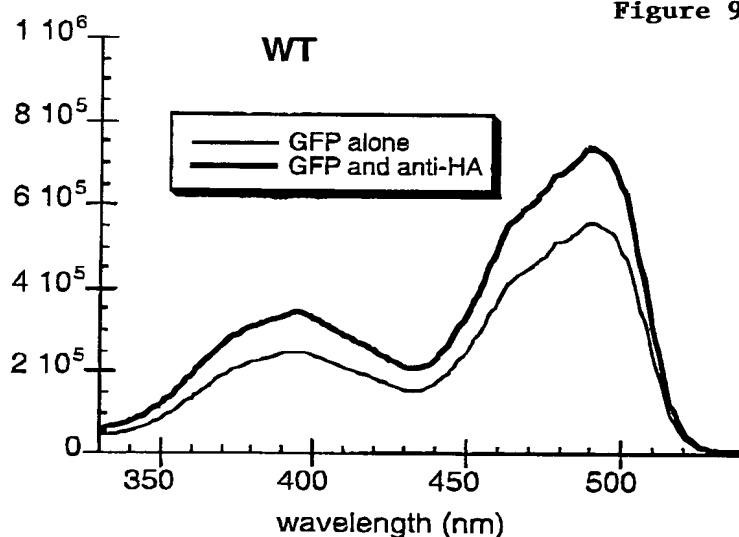


Figure 9E

Excitation spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, anti-ACT (monoclonal antibody against antichymotrypsin), and BSA were 0.025 mg/ml, 0.2 mg/ml, 0.2 mg/ml, and 0.2 mg/ml respectively. The spectra were collected at fixed emission wavelength of 550 nm. The solid thick lines represented complexes. The solid thin lines represented mutants alone. The dash lines represented mutants plus an nonspecific antibody as negative control. The dot

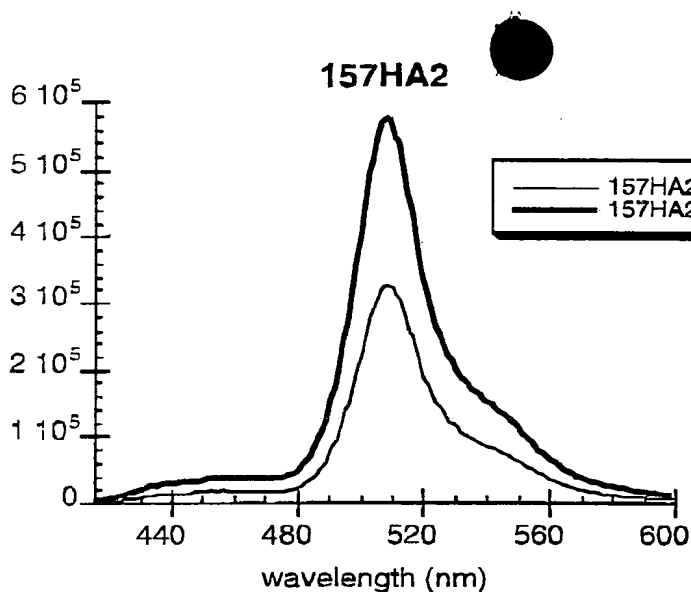


Figure 10A

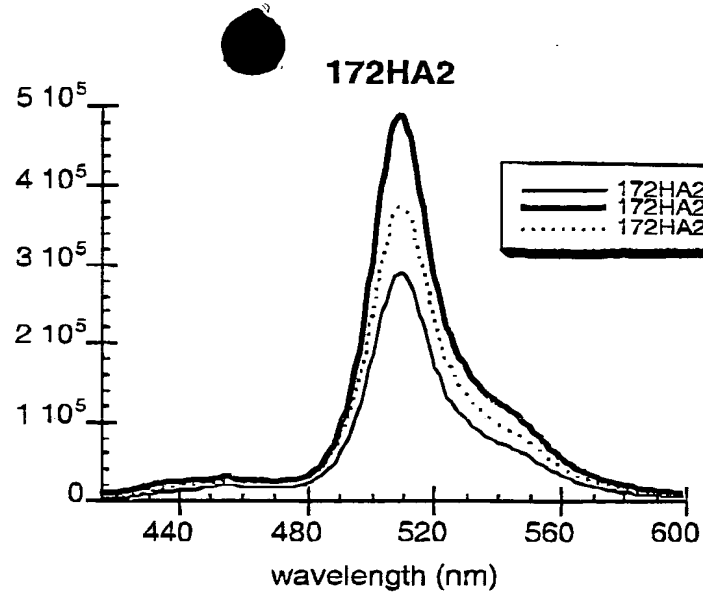


Figure 10B

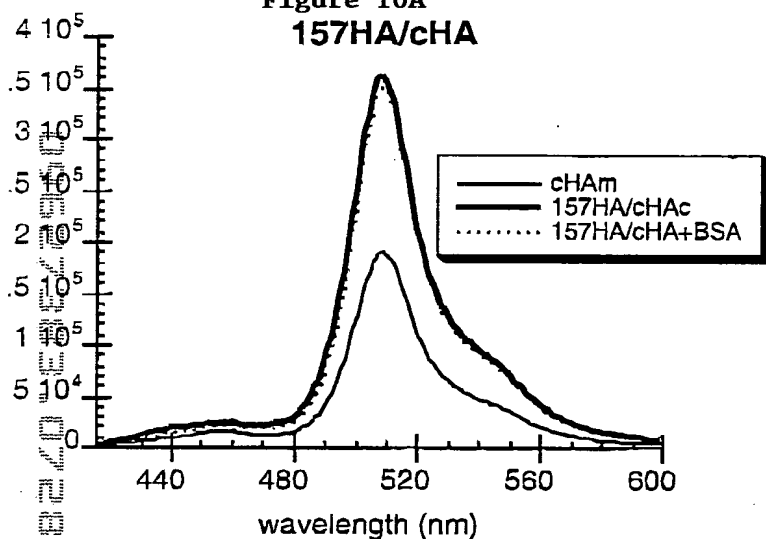


Figure 10C

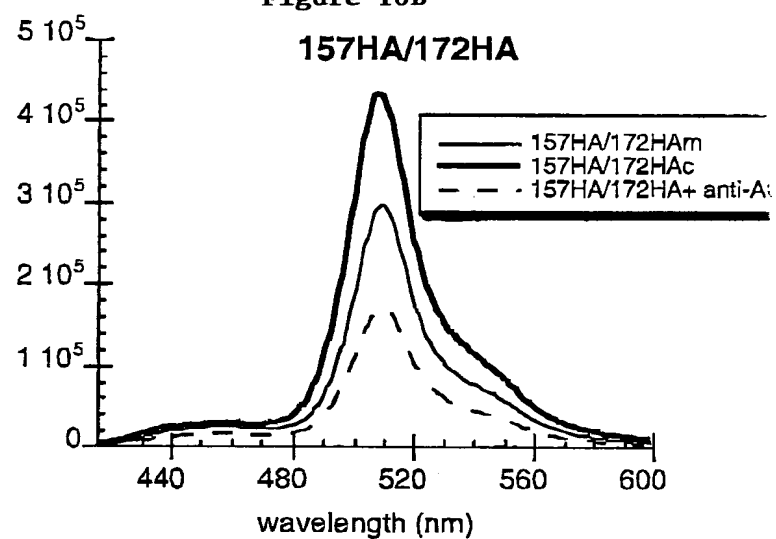


Figure 10D

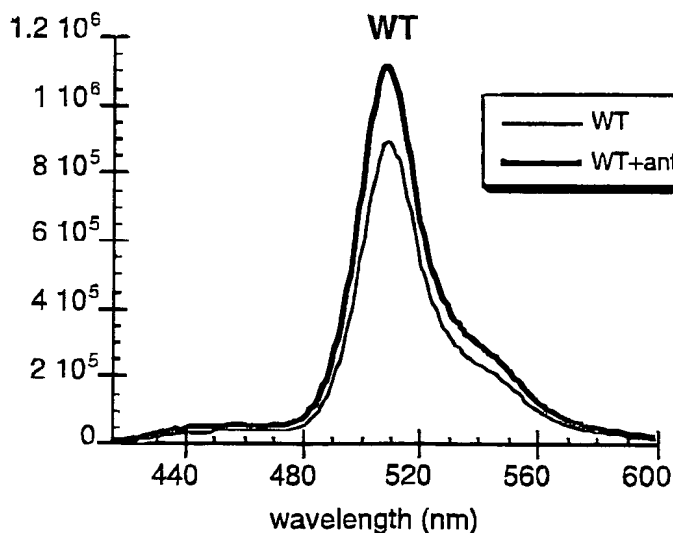


Figure 10E

Emission spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, anti-ACT (monoclonal antibody against antichymotrypsin), and BSA were 0.025 mg/ml, 0.2 mg/ml, 0.2 mg/m, and 0.2 mg/m respectively. The spectra were collected at fixed excitation wavelength of 395 nm. The red lines represented complexes. The thick lines represented mutants alone. The dash lines represented mutants plus an nonspecific antibody as negative control. The dot lines

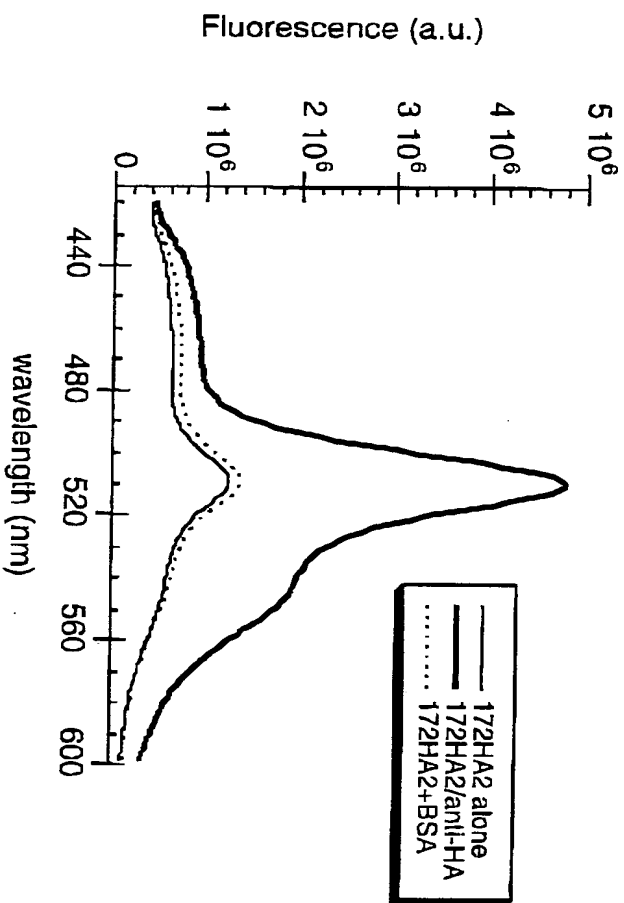


Figure 11

Emission spectra for the 172HA2 on the nitrocellulose membrane after wash.
 The thin line represented 172HA2 alone. The thick line represented 172HA2/anti-HA complex.
 The dot line represented 172HA2 with BSA.